National Cancer Institute

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CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Advancing Protein Science for Personalized Medicine

eProtein

Letter from the Director

Dear Colleagues,

This past November, the National Cancer Institute (NCI) and the National Heart, Lung, and Blood Institute (NHLBI) teamed up with the American Association of Clinical Chemistry (AACC) for a two-day meeting entitled, "Translating Novel Biomarkers to Clinical Practice: Role and Opportunities for the Clinical Laboratory." Speakers hailed from federal funding and regulatory agencies, the private sector, and the clinical chemistry community to discuss the FDA approval process and strategies for bringing clinical protein-based multiplex diagnostics to market.

The key takeaway message from the meeting was that if clinical proteomics is to be successful, the field must excel at three distinct goals: an understanding of the biological function of a given biomarker candidate, a well-defined clinical utility for that biomarker, and encouraging the adoption of diagnostic tests by clinical practitioners. However, to even begin to study the variability of biology, we need to first understand the variability of our methods and technologies. The uniformity of performance cannot be achieved without access to common performance standards, analysis of standard operating procedures (SOPs), reference to historical analytical reference data, and data standards. These standards are what the Clinical Proteomic Technology Assessment for Cancer (CPTAC) Network is generating, and they are critical to quality proteomic efforts. ▪

The 3rd Annual CPTC Meeting

Clinical Proteomic Technologies for Cancer (CPTC) held its third annual meeting in Bethesda, MD, on October 5-7, 2009, bringing together more than 200 participants—twice as many compared to last year—representing the full gamut of scientific organizations that make up the CPTC community.

The meeting included a number of talks and posters featuring innovative research being conducted

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Empowering the Scientific Community with Quantitative Tools to Measure All Human Proteins

Feasibility Study Launched

The Human Genome Project accelerated our understanding of the molecular basis of disease and these advances are slowly beginning to change the way we diagnose and treat patients. But genomic studies alone cannot capture the complete view of disease processes—a more comprehensive approach is needed.

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A Clinical Proteomic Technologies for Cancer initiative publication that builds connections throughout the proteomics community

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The 3rd Annual CPTC Meeting

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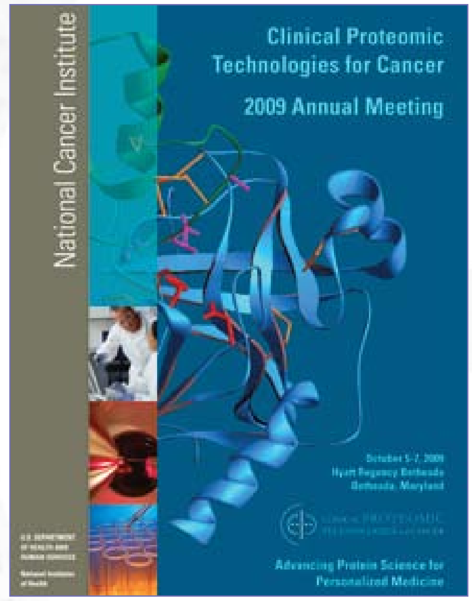
by CPTC, a program distinct through its synergistic combination of team science, individual investigators, and resource development. Of particular interest were the results of a series of successful Clinical Proteomic Technology Assessment for Cancer (CPTAC) Network experiments aimed at building a more reliable and efficient biomarker pipeline using metrics and verification technologies (quantitative mass spectrometric MRM assays).

Elda Railey, co-founder of the Research Advocacy Network, opened the meeting by reminding attendees that all efforts should be centered around the patient. Railey noted that since the CPTC initiative was first conceived in 2002, 3.9 million people have died of cancer and another 1.4 million people will be diagnosed this year, providing a sense of urgency to the work being accomplished. "The public believes in a cure," stated Railey, who concluded by reminding attendees that the advocacy community is counting on clinical proteomics researchers to bring their scientific advances to patients soon.

But scientific advances will never reach patients without volunteers for clinical trials. Dixie Mills, M.D., a renowned breast cancer surgeon and Medical Director of the Dr. Susan Love Research Foundation, discussed how the Love/Avon Army of Women is testing a new model for partnering women and scientists to find the cause and prevention of breast cancer. The goal of this revolutionary initiative is to recruit 1,000,000 women to participate in clinical research.

Keynote addresses were delivered on each day. The first day's keynote, delivered by James Heath, Ph.D., of the California Institute of Technology, focused on the integration of nanotechnology with proteomics for patient benefit. The second day's keynote, delivered by Leigh Anderson, Ph.D., of the Plasma Proteome Institute, focused on building a bridge in the biomarker pipeline between biomarker discovery and the clinical laboratory. Lastly, John Koomen, Ph.D., of H. Lee Moffitt Cancer Center & Research Institute, discussed the opportunity to incorporate quantitative mass spectrometry in the assessment of cancer patients to understand how cancer biology changes over time.



Giving a sense of the links between CPTC and other technology focused initiatives supported by NCI, the third day of the meeting was held jointly with members of NCI's Innovative Molecular Analysis Technologies (IMAT) program.[1] Several talks featured technologies and techniques developed by IMAT-supported investigators that have subsequently been applied to projects supported by CPTC, highlighting the importance of integrated technology development.

Since the CPTC initiative was first conceived in 2002, 3.9 million people have died of cancer and another 1.4 million people will be diagnosed this year, providing a sense of urgency to the work being accomplished.

In his closing remarks, CPTC Director Henry Rodriguez, Ph.D., M.B.A., noted that the initiative has made significant progress at its three-year milestone—the introduction of discovery-stage metrics; the delivery of a key milestone study demonstrating for the first time inter-laboratory reproducibility for quantitative mass spectrometric MRM assays; the development of international proteomic data release policies (Amsterdam Principles); the delivery of a caBIG® silver compliant data sharing network (Tranche developed at the University of Michigan); and the delivery of greater than 70 highly-characterized monoclonal antibodies as part of its Reagents & Resources component. These accomplishments prove that team-based science is very successful for the proteomics community and is accelerating the impact the field will have on reducing the burden of suffering and death due to cancer. ▪

[1]To learn more about IMAT, visit the program website at *http://imat.cancer.gov.*

Empowering the Scientific Community with Quantitative Tools to Measure All Human Proteins

Feasibility Study Launched

(continued from cover)

The next frontier in personalized medicine, analyzing the human proteome, might be getting a bit closer, thanks to $4.8 million in federal stimulus funding from the National Cancer Institute (NCI). This important grant was awarded to Amanda Paulovich, M.D., Ph.D., a geneticist and oncologist at the Fred Hutchinson Cancer Research Center (FHCRC) who will be co-leading a pilot study with Steven Carr, Ph.D., a senior scientific leader in protein biochemistry and proteomics at the Broad Institute in Cambridge, MA, to assess the feasibility and scalability of a method for measuring all of the proteins in the human body. The long-term output of the project will hopefully be the human Proteome Detection and Quantitation (hPDQ) project, akin to the Human Genome Project that mapped out all 20,000+ genes in the human body.

Currently there is no good way to simultaneously measure large numbers of human proteins, which severely hinders biomedical research, including the development of new diagnostics.

"Currently the biomedical research enterprise is severely hindered by its inability to measure the vast majority of human proteins."

"You can't study what you can't measure," says Paulovich. "Currently the biomedical research enterprise is severely hindered by its inability to measure the vast majority of human proteins."

This study is designed to change that. "This pilot has the potential of developing the first step toward making the entire human proteome clinically accessible," says Henry Rodriguez, Ph.D., M.B.A., director, Clinical Proteomic Technologies for Cancer (CPTC). If successful, this pilot will have a profound impact on healthcare costs and outcomes.

For the pilot, Paulovich and colleagues will use a highly sensitive emerging technology based on multiple reaction monitoring mass spectrometry (MRM-MS) to develop assays that will measure the levels of 200 proteins found in breast cancer cells. The strength of this technology is that it will enable researchers to develop multiplexed assays, which can measure large numbers of proteins in complex biological specimens simultaneously.

Unlike traditional mass spectrometry, which attempts to detect all proteins in a biological sample in a scattershot fashion, MRM-MS is highly targeted, allowing researchers to calibrate the equipment to specifically look for peptides, or protein fragments, of interest, filtering out the rest as white noise.

This project remains distinct from and complementary to existing initiatives such as the Human Proteome Project of the Human Proteome Organization (HUPO) and the Swedish Human Proteome Resource (HPR). These groups aim to identify proteins expressed in different cell types, whereas this pilot aims to quantify the expression of these proteins to see if their levels change in relation to disease.

The success of this project—the development of a robust, economical, and widely diffused capability to measure all human proteins—will stimulate a larger international endeavor that will assess the utility of all human proteins as biomarkers in hundreds of diseases and biological processes in the most efficient way.

"If we can create ways to measure a large fraction of human proteins, particularly those in very low abundance, this will facilitate the development of new drugs and personalized medicine," Paulovich said. ▪

For more information, please click on the following:

Press release

NIH Reporter

Amanda Paulovich, M.D., Ph.D.

Steven Carr, Ph.D.

Incentivizing Proteomic Data Sharing

Beginning January 2010, authors who publish a manuscript containing mass spectrometry data in *Molecular and Cellular Proteomics* (MCP) must submit the raw data on a publicly accessible site by the time of publication. The revised guidelines released by MCP, "Revised Publication Guidelines for Documenting the Identification of Peptides, Proteins, and Post-Translational Modifications by Mass Spectrometry," are the first of their kind to make the sharing of raw data mandatory if a manuscript is to be accepted for publication. Other scientific journals only strongly recommend such practices. Will MCP's efforts lead the way towards a new industry standard?

The MCP 2005 Paris guidelines for data submission and quality encouraged authors to deposit their data in public repositories. "We did not insist on the use of those repositories simply because of the difficulties involved in actually using them," explains Steve Carr, Ph.D., Director of Proteomics, Broad Institute, member of the Clinical Proteomic Technology Assessment for Cancer (CPTAC) Network, and associate editor of MCP. What transpired over the past few years are substantial improvements in the ease with which one can upload data to these sites and a continuing explosion in the generation of large proteomic datasets.

These advancements were the impetus for a recent summit held in Amsterdam where the international proteomics community began defining policies and practices that would govern and facilitate the release of proteomic data into the public domain. "The meeting in Amsterdam got people within the community to start thinking about how practical data sharing actually is and what the issues may be. It was certainly good groundwork for us," explains Bradshaw.

The outcomes of this summit, the Amsterdam Principles, proposes that raw data—straight from the mass spectrometer—would be the best type of data to share.

"We're really trying to capture the raw data itself, the unprocessed data. At the moment, Tranche is the only resource out there that allows you to do this," explains Karl Clauser, Ph.D., Research Scientist, Broad Institute. However, MCP is not requiring authors to deposit data in Tranche. Any public repository is acceptable as long as it is independent of the authors' control.

The benefits of making raw proteomic data publicly accessible to the scientific community include improved algorithm development for software that can accurately identify peptides and proteins in complex samples, quality control of the data submitted, and reuse of data by other investigators.

"The ability to have access to the raw data and be able to assess that information for yourself and decide whether you believe the author's interpretation is certainly very important," explains Ralph Bradshaw, Ph.D., Professor, University of California, San Francisco and co-editor of MCP.

MCP is taking the lead in this endeavor because they realize there is an incentive for investigators to deposit data if it is coupled with the ability to publish their manuscript. In fact, data deposition may ultimately enhance a researcher's reputation.

It is up to the entire community to take the necessary steps to ensure that all data be made publicly available.

"Researchers who deposit data sets that subsequently prove particularly useful to the community would end up with highly cited data, and could thereby be rewarded accordingly," explains Carr. This could provide greater incentive than the present system of evaluation, which is skewed almost exclusively to publications in high-profile journals and citation metrics.

To fuel progress in proteomics research, data sharing cannot be voluntary; rather, it is up to the entire community to take the necessary steps to ensure that all data be made publicly available. MCP should be applauded for their efforts as the release of such data will put the pace of proteomic research on a trajectory similar to that seen in genomics. ■

Tranche: Reducing Barriers to Proteomic Data Sharing

Researchers in the field of proteomics generate vast quantities of raw data with every experiment conducted, and these data sets, which tend to be global protein analyses looking at very large numbers of proteins, could be reused in various ways. For example, data sets from multiple breast cancer studies could be aggregated and—with greatly increased statistical power—uncover entirely new cancer biomarkers that are present at very low levels in patient samples. Data sets generated to measure protein levels in one particular study could now be mined by a different group of researchers who are analyzing modified proteins. Also, access to multiple data sets would allow software developers to generate new algorithms that identify peptides and proteins in complex samples with much greater confidence.

Although the value of making proteomics data accessible to the greater biomedical community cannot be argued, developing methods to implement data exchange has been a significant challenge. In fact, researchers have had to mail computer hard drives to collaborators in order to share large mass spectrometry data files. Such a process is obviously inefficient and the number of users who can access the data is severely limited.

"We felt that there must be people wanting to put those data sets out in the public domain, but the problem was that it was a difficult thing to do," explains Phillip Andrews, Ph.D., Professor of Biological Chemistry and Bioinformatics at the University of Michigan, and a member of the Clinical Proteomic Technology Assessment for Cancer (CPTAC) Network.

Andrews and colleagues overcame this obstacle through the development of Tranche, a free and open file sharing tool that is used extensively by the CPTAC Network. Tranche is currently the only repository that can store very large proteomic raw data sets, and all public data sets are citable in scientific journals.

Tranche is structured as a peer-to-server-to-peer (P2S2P) distributed network to provide simplified access to data, quality assurance about data integrity, as well as security. Researchers can simply search for data sets using unique ID hash codes

> *"We felt that there must be people wanting to put those data sets out in the public domain, but the problem was that it was a difficult thing to do."*

and then download the data on to their computers for analysis.

"Tranche is more than just a data sharing tool between individual researchers. It's a data dissemination system," explains Andrews. Since its launch in 2006, Tranche now has more than 400 registered users and is hosting 11 terabytes (TB) of proteomics data across 17 servers located in the U.S. and Japan.

"Tranche is being used by most other proteomic data resources as a source of data, including the PRoteomics IDEntification database (PRIDE), the PeptideAtlas, and the Global Proteome Machine Database (TheGPMdb). So once you deposit data in Tranche, it will end up in these other resources," explains Andrews.

Two major developments have been made this year that significantly improve both the usability and accessibility of Tranche: ProteomeCommons.org and caBIG® compatibility.

ProteomeCommons.org is a public database created by Andrews that is linked to the Tranche data repository. Containing an online project management tool, this site provides researchers access to free, open-source proteomics tools and resources.

"ProteomeCommons is a collaboration tool that's based on a social networking model. You click a button to start a new

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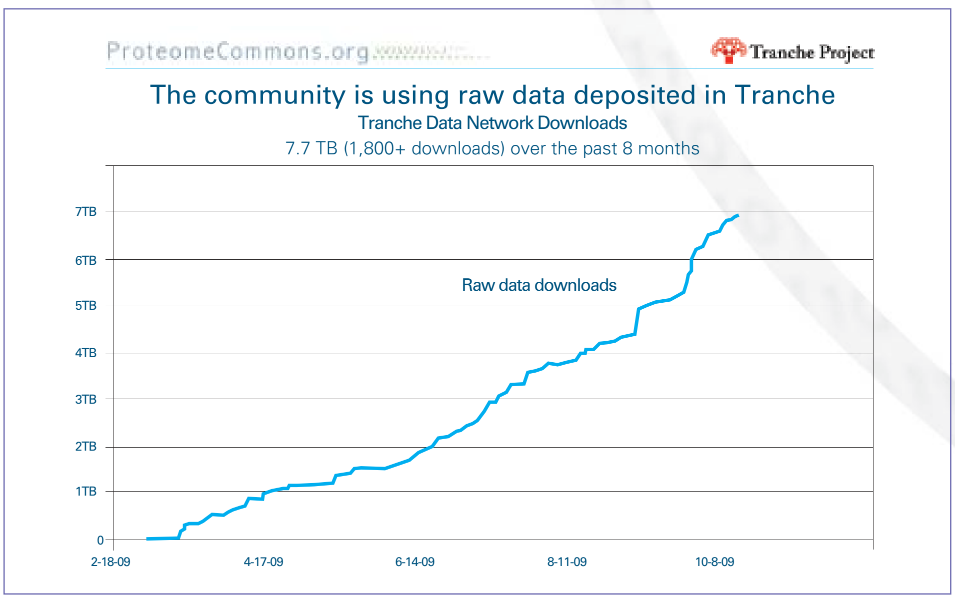

An Advocate's Perspective:

Hard Hats Required: A Blueprint for the Cancer Research Community

Paula Kim

Paula Kim, Founder, Chair, and Chief Executive Officer of Translating Research Across Communities (TRAC), a strategy firm that works to advance patient-centered research and care by coordinating the collective goals of the many stakeholders in the cancer research enterprise, understands the need for a structurally sound foundation and clear plan if the true potential of new research in cancer is to be realized.

For Kim, the need for a strong foundation and articulated plan, or "blueprint," are natural carryovers from her experience in the world of construction. She draws on this background in her work throughout the National Cancer Institute (NCI), where she has served on the Board of Scientific Advisors, worked with the Office of Biorepositories and Biospecimen Research (OBBR) as it was being developed, and also consulted with Clinical Proteomic Technologies for Cancer (CPTC).

"My background in construction," says Kim, "actually helps me to track and follow multiple paths and activities in these complex life sciences research areas and translate the work in such a way that patients, policymakers, benefactors, and advocates, understand the potential relevance for them and their loved ones."

In addition to her work in construction, Paula Kim also draws on her personal experiences with cancer to motivate and strengthen her commitment to advocacy. In 1998, Kim's father was diagnosed with pancreatic cancer, and he lost his battle seventy-five days later. Called to action by this tragedy, Paula co-founded the Pancreatic Cancer Action Network (PanCAN) in 1999, the first national patient advocacy organization for pancreatic cancer, serving as Founding Chairman of the Board, then Chief Executive Officer and President. In 2004, she resigned from the organization and moved forward to establish TRAC and Paula Kim Consulting.

As Kim became more involved in the cancer research community and more aware of cutting-edge technologies and research being done at NCI, such as the proteomics research conducted by researchers in the CPTC network, she began to realize how these complex technologies needed to be translated and made relevant for the other stakeholders in the cancer research community (policymakers, advocates, patients, etc.) before their full potential could be realized. She believes that getting people to understand the potential and relevance of these complex technologies is one of the largest roadblocks facing NCI today.

For Kim, advancing cancer research requires two key elements: translation and also a well-defined, universally understood approach to organizing and conducting the work that researchers are pursuing throughout the NCI. In construction, there is a universal categorization and organizational system; Kim can walk onto a construction site anywhere in the world and follow the blueprint because of the standard system and set of clearly defined plans in place. Kim used this mapping approach to develop the Pancreatic Cancer Research Map, a clearinghouse Web site that provides a comprehensive list of investigators and research projects relevant to pancreatic cancer, organized in relation to facilitate key priorities defined by NCI's pancreatic cancer Progress Review group in 2001. The site facilitates collaborations among researchers in this field, and that provides information for funders, advocates, and patients as well.

Kim believes that proteomics is a field that could benefit from this combined translation/mapping effort. According to Kim, "Proteomics has a great deal of potential but also a great deal of complex obstacles to overcome." CPTC has the opportunity, through its extensive network of Clinical Proteomic Technology Assessment for Cancer (CPTAC) teams and its role as a leader in proteomics research, to serve as a hub of information not only for proteomics researchers but also for patients, advocates, and policymakers who want to learn more about this fast-developing area of cancer research. CPTC can "connect the dots," says Kim providing educational resources and building inroads for the public into the dynamic world of proteomics research. ■

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In Vitro Diagnostic Tests for Cancer: Regulation of Laboratory Developed Tests

Jeffrey N. Gibbs
Director
Hyman, Phelps & McNamara, PC
Washington, DC

Given that many protein-based assays do involve a calculation derived from multiple markers, the IVDMIA policy, if adopted, may have a particularly significant impact on this sector.

There are two principal routes by which new protein-based assays enter the commercial market. As described in the first of this two-part series, one route is to obtain clearance or approval from the Food and Drug Administration (FDA); the second route is to offer the assay as a laboratory developed test (LDT).

LDTs have long been an important vehicle for the introduction of new assays. This was true before Congress enacted the Medical Device Amendments of 1976, which greatly expanded FDA's authority over devices, and it remains the case today. For example, while FDA has reviewed only a handful of genetic tests, it is estimated that over 1,300 genetic tests are being offered by laboratories.

Regulation of LDTs

LDTs are actively regulated, at both the federal and state levels. Under the Clinical Laboratory Improvement Amendments (CLIA), laboratories must meet a number of requirements imposed by the federal government and enforced by the Centers for Medicare & Medicaid Services (CMS), which include personnel qualifications, training, recordkeeping, documentation, and procedures. Laboratories must be registered or licensed in order to offer diagnostic tests and are subject to inspection. Some states, such as New York and California, require that laboratories be licensed by state agencies before any patient from that state is tested. A number of states also have separate laws relating to genetic testing and to "direct access testing," i.e., offering tests directly to consumers. Laboratories need to comply not only with CLIA but with this array of state laws. In addition, laboratories may need to perform proficiency testing which is administered by various third party groups. Not all types of testing, however, are subject to meeting proficiency testing.

Other regulatory bodies also play an oversight role. The Federal Trade Commission (FTC) has jurisdiction over claims made regarding products and services offered in the U.S. and has the authority to take action against laboratories that make claims the FTC believes are false or misleading. States also have their own regulatory bodies that can act against false or misleading claims by laboratories.

Although laboratories are heavily regulated, one perceived gap in the regulatory system has received much attention: clinical validation. Critics have asserted that laboratories are not obligated under CLIA to clinically validate their assays. Whether that is a correct reading of the law has been hotly debated, but CMS has stated that it does not have the power to ensure that assays are clinically valid. In a partial response to this perceived lacuna, FDA has stepped up its role in regulating LDTs.

FDA and LDTs

The Medical Device Amendments of 1976 defines the term "device" as including an "*in vitro* reagent" that is "intended for use in the diagnosis of disease"; laboratory tests are not specifically mentioned or excluded. From 1976 to 1992, there was no indication that FDA intended to play any role in regulating laboratories. Then in 1992, FDA asserted that LDTs were "devices" and therefore subject to FDA regulation. That position has been controversial, but FDA has repeatedly reaffirmed this view, while still saying that it would generally not regulate LDTs. Within the past few years, though, FDA has begun to play a much more active role in regulating LDTs.

The single most significant FDA initiative was the issuance in September 2006 of a draft policy aimed at In Vitro Diagnostic Multivariate Index Assays (IVDMIAs). IVDMIAs were defined as assays that took the results from two or more markers and calculated a single index score. FDA said that these assays would need to meet the statutory requirements that apply to medical devices, and the draft IVDMIA policy received heavy criticism. FDA released a revised version in 2007, which also elicited a number of negative comments and recommended modifications. To date, the draft policy has not been adopted. Given that many protein-based assays do involve a calculation derived from multiple markers, the IVDMIA policy, if adopted, may have a particularly significant impact on this sector.

In the meantime, FDA has taken action against individual assays offered by laboratories. The agency has also begun to question companies that supply the tools used by laboratories to develop their LDTs.

It remains unclear what role FDA will play in the future in regulating protein-based LDTs, but it is very likely that it will be a much greater role than has historically been the case. ■

Investigator Spotlight: *Michael MacCoss, Ph.D., University of Washington*

Skyline: Building a Bigger Net for Targeted Proteomics

Michael MacCoss, Ph.D.

Biomarker discovery using current proteomic technologies generates a lengthy list of candidates, most of which are false positive. Culling through this list to identify the most promising clinically-relevant biomarkers for further validation is the biggest rate-limiting step in protein biomarker research. To overcome this bottleneck, targeted proteomic technologies are needed that accurately and efficiently credential biomarker candidates prior to clinical validation.

A huge emphasis within the Clinical Proteomic Technologies Assessment for Cancer (CPTAC) program is developing technologies for targeted proteomics. Recently, CPTAC researchers demonstrated that an emerging technology for detecting and quantifying protein biomarkers in body fluids, based on selected reaction monitoring mass spectrometry (SRM-MS), may ultimately make it possible to screen large numbers of biomarker candidates in hundreds of patient samples, thus ensuring that only the strongest biomarker candidates will advance into clinical validation.

One difficulty in making such technology routine is the complexity and labor involved in producing an optimized instrument method that measures many target peptides in a single analysis. This was largely a manual process until Michael MacCoss, Ph.D., Associate Professor of Genome Sciences, University of Washington, decided it was time for a new approach.

MacCoss recruited professional software engineer, Brendan MacLean, to develop a software tool that would greatly shorten the path to a fully optimized instrument method. "Skyline is software that's designed to help people build instrument methods and analyze their mass spectrometry data when they are doing a targeted proteomics experiment," explains MacCoss.

"When I first started, it was a lot of effort to set up an instrument to measure just a few peptides. You couldn't measure 500 peptides and figure out whether one of them is having some effect. Increasing the size of that net is a big part of what Skyline is about," explains MacLean.

Brendan MacLean

"The CPTAC Network is using it pretty intensely, and it's allowing those labs to share all of this information. Investigators are excited about that."

Skyline is one of the few instrument software programs for proteomics that can write methods and read input data in a vendor-neutral manner. For example, it becomes very easy for an investigator who is using a particular vendor's mass spectrometer to share their data with someone who does not have that instrument or the accompanying software. "The Clinical Proteomic Technology Assessment for Cancer (CPTAC) Network is using it pretty intensely, and it's allowing those labs to share all of this information. Investigators are excited about that," explains MacLean.

All efforts are in vain, however, without community adoption. The key to success for any software program is to make it as user-friendly as possible. For this reason, both software installation and updates are a largely automatic process. In addition, three short instructional videos have been produced that walk the user through each step in the process using examples. Word of mouth marketing alone has resulted in several hundred investigators downloading the software. MacCoss expects a significant surge in new users following publication of the first Skyline manuscript, which is currently in preparation. ■

For more information about Skyline please visit: http://proteome.gs.washington.edu/software/skyline.

Investigator Spotlight: *Susan Fisher, Ph.D., University of California, San Francisco*

Leveraging the Proteome's Complexity for the Early Detection of Aggressive Breast Cancer

Susan Fisher, Ph.D.

The biggest challenge inherent in clinical proteomics lies in the protein's increased degree of complexity compared to its corresponding gene. For example, one gene can encode more than one protein due to a process known as alternative splicing. Splicing explains why the human genome contains approximately 21,000 protein-encoding genes, but the total number of proteins is estimated to be between 250,000 to one million. Proteins are continually undergoing changes such as binding to a cell membrane, partnering with other proteins, or breaking into two or more pieces. Finally, proteins can be chemically modified shortly after or even during synthesis in a process known as posttranslational modification (PTM). These modifications may extend or control the range of functions carried out by the protein.

As a result of this complexity, proteins may vary considerably from one person to another, under different environmental conditions, or even within the same person at different ages or states of health. Susan Fisher, Ph.D., University of California, San Francisco, and colleagues are looking closely at these protein splice variants and PTMs—specifically, glycosylation, phosphorylation, and modifications that are indicative of oxidative damage—because many are proving to be associated with cancer. Proteins carrying cancer-associated modifications may ultimately serve as biomarkers for the early detection of disease.

"We are concentrating on biology-driven biomarker discovery with a focus on proteins that are secreted by breast cancer cells," said Fisher. Methods are being developed and implemented to detect splice variants and PTMs in patient samples with the goal of developing blood-based diagnostic tests that can be used in the clinic. Specifically, the UCSF team is identifying biomarkers that can detect metastatic-prone (basal subtype) breast cancers early, before the cancer has spread. Biomarkers such as these are likely to have the greatest impact on survivorship in the clinic.

Many genes have been identified to date that show strong evidence of alternative splicing in breast cancer cells. "Together with Dr. Joe Gray at Lawrence Berkeley National Laboratories, we have constructed assays that allow us to quantify new protein variants that result from alternative splicing," said Fisher. These variants are now being tested in plasma from breast cancer patients.

In complementary efforts, the UCSF team and Dr. Brad Gibson from the Buck Institute for Age Research are analyzing the same subset of aggressive breast cancer cells for unusual glycosylation patterns because malignant cells are known to release glycosylated proteins into the circulation that carry disease-related carbohydrate epitopes. "Breast cancers up-regulate the expression of carbohydrate structures that play a role in the immune system, but that are also relevant to metastasis. We are using this knowledge to formulate new methods for capturing these glycosylated proteins from blood in breast cancer patients," explains Fisher.

They have already identified a potential biomarker candidate—a glycosylation variant that may be involved in the metastatic process and is thus of high diagnostic value. This candidate is under intense investigation by the UCSF team as a potential biomarker for the early detection of aggressive breast cancer.

> *Biomarkers such as these are likely to have the greatest impact on survivorship in the clinic.*

"We are incredibly enthusiastic about this program and believe in the approach this program is taking. It is gratifying to see the progress that has already been made by the UCSF team," said Fisher. ■

Tranche: Reducing Barriers to Proteomic Data Sharing

(continued from page 5)

project, invite investigators who you want to collaborate with, and they receive an email asking them to join," explains Andrews. Researchers can keep track of every data set that has been uploaded to Tranche and can see how many times each data set has been used by other researchers.

In addition, an annotation tool allows researchers to annotate the data as soon as they're uploaded to Tranche. In September 2009, these annotations were made accessible to caGRID and Tranche completed its silver level caBIG® compatibility review. By connecting Tranche to caGRID, the detailed proteomic data sets are readily accessible by caBIG® researchers. These detailed data sets are extremely valuable in the search for clinically-relevant cancer biomarkers. ■

For more information about Tranche, please visit: https://trancheproject.org/
For more information about ProteomeCommons, please visit: https://proteomecommons.org/

Industry News

2008-2009 CPTC Annual Report Now Available

The 2008-2009 Clinical Proteomic Technologies for Cancer (CPTC) Annual Report chronicles the past two years of the initiative's efforts, offering highlights and progress reports from each of the CPTC programs, as well as information about the collaborative community and public/private partnerships that are a large part of CPTC.

Newly Expanded CPTC Reagents Data Portal!

The CPTC Reagents Data Portal, which serves as a central source of reagents and resources made available by the CPTC initiative for the scientific community, is rapidly expanding as the initiative makes way for numerous reagents and resources in the pipeline that are greatly needed for effective proteomic analysis. Come visit the newly revamped portal!

First-of-its-Kind Yeast Standard Promises to Optimize LC-MS/MS Performance and Generate Higher Quality Proteomic Data

A recent collaborative study between CPTC and the National Institute of Standards and Technology (NIST) has generated promising results on a yeast performance standard using Standard Operating Procedures (SOP) and metrics. This work, published in *Molecular and Cellular Proteomics*, provides a basis for laboratories to benchmark their own performance, improve upon current methods, and evaluate new technologies. Additionally, the researchers demonstrated the utility of the yeast reference (spiked with human proteins) to benchmark the power of proteomic platforms for detection of differentially expressed proteins at different levels of concentration in a complex matrix, providing a metric to evaluate and minimize pre-analytical and analytical variation in comparative proteomics experiments.

An Automated and Multiplexed SISCAPA-MRM-based Quantification Method for Protein Biomarkers

A recent study led by Dr. Amanda Paulovich from Fred Hutchinson Cancer Research Center described significant advances made to the current SISCAPA (Stable Isotope Standards with Capture by Anti-Peptide Antibodies) technology, published in *Molecular and Cellular Proteomics*. By creating an automated magnetic bead-based platform for multiplexed SISCAPA assay coupled with MRM (Multiple Reaction Monitoring) mass spectrometry (nine targets in one assay), these scientists demonstrated that this high-throughput assay can detect proteins in the physiologically relevant concentration range (low ng/ml from 10 µl plasma) with sufficient precision (median CV 12.6%) by enriching peptides of interest from large volume of plasma. This study serves as a critical step in the long path of translating multiplexed proteomics technology into clinical laboratories for personalized medicine.

New Software Suite Constantly Monitors Performance Metrics for Liquid Chromatography-Tandem Mass Spectrometry Systems in Proteomic Analyses

In an attempt to address variability issues commonly associated with mass spectrometry (MS)-based proteomic analysis, the National Institute of Standards and Technology (NIST) developed 46 system performance metrics for monitoring chromatographic performance, electrospray source stability, MS signals, dynamic sampling of ions for MS/MS and peptide identification. Supported in part by the CPTC initiative, these metrics typically display variations less than 10% and thus can reveal subtle differences in performance of system components. Application of these metrics enables rational, quantitative quality assessment for proteomics and other LCMS/MS analytical applications. This study, published in *Molecular and Cellular Proteomics*, lays the foundation for the standardization of various MS-based platforms to ultimately support the assessment of proteomic differences between biologically interesting samples.

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Advancing Protein Science for Personalized Medicine

Upcoming Events

February 3-5, 2010
Molecular Medicine Tri-Conference 2010
Organized by:
Cambridge Healthtech Institute
San Francisco, CA

February 6-10, 2010
Mass Spectrometry: Applications to the Clinical Laboratory 2010
Organized by:
MSACL
San Diego, CA

February 25-26, 2010
5th Annual Biomarkers Congress
Organized by:
Cambridge Healthtech Institute
Manchester, UK

March 7-10, 2010
Proteomics from Bench to Clinic
Organized by:
US HUPO
Denver, CO

For a full list of upcoming events, visit http://proteomics.cancer.gov/mediacenter/events.

Contact Information

For more information about the CTPC, please visit http://proteomics.cancer.gov, or contact us at:

National Cancer Institute
Office of Technology & Industrial Relations
ATTN: Clinical Proteomic Technologies for Cancer
31 Center Drive, MSC 2580
Bethesda, Md 20892-2580
Email: cancer.proteomics@mail.nih.gov

The NCI Clinical Proteomic Technologies for Cancer initiative seeks to foster the building of an integrated foundation of proteomic technologies, data, reagents and reference materials, and analysis systems to systematically advance the application of protein science to accelerate discovery and clinical research in cancer.

Reagents Data Portal

http://antibodies.cancer.gov
http://dshb.biology.uiowa.edu

Newly Released Antigens and Antibodies

Antigen	Antibody
14-3-3 Sigma	CPTC-SFN-1 CPTC-SFN-2 CPTC-SFN-3
BCL2-like 2	CPTC-BCL2L2-1 CPTC-BCL2L2-2 CPTC-BCL2L2-3
Calcyclin	CPTC-Calcyclin-1 CPTC-Calcyclin-2
Chloride Intracellular Channel 1	CPTC-CLIC1-1 CPTC-CLIC1-2
Fascin	CPTC-Fascin-1 CPTC-Fascin-2 CPTC-Fascin-3
Glutathione S Transferase M1	CPTC-GST M1-5 CPTC-GST M1-6 CPTC-GST M1-7
Melanoma Antigen Family A, 4	CPTC-MAGEA4-1 CPTC-MAGEA4-2 CPTC-MAGEA4-3
MethylCpG Binding Protein 1	CPTC-MBD1-1 CPTC-MBD1-2 CPTC-MBD1-3
Protein Phosphatase 2A	CPTC-PP2A-1 CPTC-PP2A-2 CPTC-PP2A-3 CPTC-PP2A-4
Ubiquitin conjugating enzyme E2C	CPTC-UBE2C-1